(1) INVESTIGATION OF METHODS OF ASHING AND DISTILLATION PRIOR TO THE DETERMINATION OF FLUORINE IN ORGANIC MATERIALS, and (2) THE THERMODYNAMICS OF THE WOOL KERATIN-WATER VAPOUR SYSTEM

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ABSTRACT

Section 1

Studies have been carried out on a simplified distillation technique and alkaline fusion in the colorimetric determination of fluoride in organic materials. A number of teeth have been analysed as a test of the efficiency of the procedure developed. On the basis of the results obtained it has been concluded that the distillation is unsatisfactory since incomplete recovery with a large positive blank is observed.

The indicator consisting of an acid solution of zirconylalizarin sulphonate has been modified on the basis of experiments carried out with a view towards extending the range of usefulness and accuracy of the indicator. The desired results were obtained.

Section 11

The heats of wetting by water of wool keratin initially containing various amounts of adsorbed and desorbed water have been measured using an adiabatic calorimeter. These measurements along with the water vapour sorption isotherm have been used to calculate the thermodynamics of the system. No hysteresis was observed in the heat of wetting. The differential heats and entropies obtained taken together with those for the silk fibroin-water system have led to generalizations concerning the swelling of fibrous proteins by water. The magnitudes of these properties suggest hydrogen bonding. At low

water contents it is observed that the differential heats and entropies of the system are to a much greater extent than previously realized a function of the adsorbate. A significant amount of energy is used to swell the protein at low water contents and the entropy of swelling in this region is seen to have a marked effect on the differential entropy of the system.

The adsorption areas at high water contents suggest that the water molecules form clusters rather than continuous films as on silk and cellulose.

THE UNIVERSITY OF ALBERTA

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SECTION I

INVESTIGATION OF METHODS OF ASHING AND DISTILLATION PRIOR TO THE DETERMINATION OF FLUORIDE IN ORGANIC MATERIALS

INTRODUCTION

This research is a continuation of the program initiated at the University of Alberta in 1935 on methods of determining fluoride in naturally occurring materials. In the early years it was of special significance in the mottling of the enamel but more recently the importance of the analysis is related to the fluoridation of water supplies as a means for the reduction of dental caries.

Although considerable work has been done in this project on the final determination step, the primary purpose has been that of investigating methods of ashing organic materials and studying the use of sulfuric acid as a distilling agent to replace perchloric acid which is now recommended in official methods of analysis of fluoride in organic materials (3).

Walker and Spencer (15) using the method of Sanchis (7) and a modified Armstrong method (1) determined the fluoride content of water from 254 areas of Alberta and correlated these data with the occurrence of mottled tooth enamel. The Sanchis method which is colorimetric was used for waters with low fluoride content and the titrimetric Armstrong method for those with higher amounts.

Walker and Finlay (13) investigated the application of a titration method and of a colorimetric method to the determination of small amounts of fluoride in water and made a comprehensive study of the effect of a number of interferring species.

An adaptation of the Sanchis method for use in a photometric colorimeter was made by Walker and Gainer (14). The absorption maximum of the red lake of zirconyl alizarin sulfonate was determined and a filter of 515 millimicrons chosen for use. A second photometric method employing the purple lake of aluminium-hemotoxylin was reported by Price and Walker (6).

A number of investigators under the direction of Dr. Walker (11) carried out a refinement of the photometric method using a Lumetron photoelectric colorimeter and employing the indicator described by Scott (8,8a). An accurate determination of the interferring effect of sulfate and phosphate was also carried out.

An investigation of methods of fusion for destroying organic matter in teeth, bones, and other materials prior to the determination of fluoride content was made in this laboratory by Misae Hironaka and Hinda Doz under the direction of Dr. Walker (12).

OUTLINE OF PRESENT RESEARCH

Our work was directed towards the developing of a complete procedure for the analysis for fluoride in tooth material.

Care in choice of ashing materials is necessary and the indicator was not completely satisfactory, hence these factors had to be studied. Since the distillation is, in most procedures, the longest and most tedious step it was desired to develop a method capable of accuracy to ± 0.1 ppm. but more rapid.

This is a reduction in accuracy from that of some procedures (4) but adequate for many purposes. The final procedure was to be used on the analysis of certain Eskimo teeth.

The work to be reported breaks down into four parts:

1) An investigation of the indicator with a view to extending its range of usefulness.

2) A study of the recovery in the distillation step. The method used was Thrun's (10) procedure modified largely on the basis of experience.

3) A study of various fusion materials.

4) The application of the conclusions reached to the analysis of teeth obtained from various sources.

NOTE: The term ppm as it appears can be taken to mean milligrams per 100 ml.

PART I - INDICATOR

In general the colorimetric method, whether visual or photometric, depends upon the bleaching of the lake of metallo-organic complexes. Visual methods utilizing Nessler tubes are quite accurate but depend upon the ability of the analyst's eyes to differentiate small changes in shade and tint. The subjectivity, and eyestrain, involved can be removed by the use of photometric colorimeters.

The indicator reported by Scott (8,8a) has a limited range of applicability of 0-1.6 ppm. It was desired to extend this range and, if possible, increase the accuracy. Scott pointed out that the range and shape of the % transmission curve is a function of the concentration of the constituents used. Hence it was thought possible to accomplish the desired result through studying the effect of varying the concentrations of these constituents.

Increased zirconyl-alizarin sulfonate resulted in good differentiation at higher fluoride concentration but was nearly useless at the lower range. The effect of the concentration of the acids was the reverse, i.e.:increased acid concentration gave good resolution at lower fluoride concentration but poor at the upper end of the range.

The mixed acid used consisted of equal volumes of sulfuric acid and of hydrochloric acid of equal normality. The concentrations studied varied

from 3.0N in sulfuric acid and hydrochloric acid as reported in (8) to 2.7N as in (8a) in steps of .05N.

The zirconyl-alizarin sulfonate was prepared by dissolving 0.43 gram of zirconyl chloride in 50 ml of H_2O and 0.I gram of Sodium alizarin sulfonate in a second 50 ml portion of water and then mixing the two slowly. The mixture is then allowed to stand for 15 minutes before adding the acid. Following the Scott procedure 70 ml of this mixture is then diluted to 1 liter with the mixed acids. We studied variation in which 60-85 ml of lake were used. Twenty modifications were studied in which the concentrations were varied as indicated.

Since, at the beginning of the project, the colorimeter was out of order the first nine variations were evaluated visually using Nessler tubes. After the repair of the photometric colorimeter, comparison of numbers 10-20 was made by plotting calibration curves of % transmission versus fluoride concentration.

The method of study briefly was this: 100 ml of solution containing 0-2. ppm fluoride were prepared from a standard sodium fluoride solution. To each in a 250 ml Erlenmeyer was added 5 ml of the indicator being studied. The resulting pale yellow solution was allowed to stand for one hour for color development to take place. The solutions were then placed, in turn, in a 150 mm cuvette and the % transmission determined with the Lumetron colorimeter. The machine was calibrated at 100% transmission with distilled water in the cuvette.

There are also a few important considerations regarding the effect of aging and temperature on the indicator. Previously it had been found that the indicator demonstrated a changing calibration curve over a period of months. With No. 19, the only so studied, by care in controlling the admission of sunlight and temperature changes on the stock solution of indicator it was found that the indicator was stable over a period of at least three months.

One very important factor not previously noted was the sensitivity of the indicator to the temperature at which it was used. It was found that a temperature variation of approximately 2°C from that at which it had been calibrated introduced a rather considerable error.

Also it was again verified that each new batch of indicator even if of the same concentration, required a new calibration curve since a significant variations of % transmission between batches at a given fluoride concentration are noted.

Of the 20 indicator variations studied 3 demonstrated optimum ranges of usefulness, numbers 12, 17, and 20. Table I and Figure I give the results for some of the indicators studied.

Having developed an adequate indicator it was decided to proceed to the 2nd part of the project.

TABLE I

CALIBRATION CURVES FOR VARIOUS MODIFICATIONS

% TRANSMISSION

F- added in ppm	Scott	No. 14	No. 15	No. 16	No. 17	No. 19
0.0	45.4	52.2	62.4	56.8	44.6	41.1
0.2	47.1	57.5	67.7	62.0	49.5	44.9
0.4	50.0	64.4	75.7	68.9	55.3	50.6
0.6	52.9	72.3	82.5	78.0	64.1	56.2
0.8	56.6	80.4	87.8	83.3	70.2	62.2
1.0	61.1	86.1	91.9	90.4	78.9	70.7
1.2	65.5	91.2	97.2	93.6	84.7	76.8
1.4	70.0	96.1	96.9	96.8	90.4	86.4
1.6		98.5	98.0	98.3	93.0	89.4
1.8			98.5	98.6	96.8	91.2

NOTE: The concentrations of reagents used in the indicator modifications of Table I are as follows: the mixed acids were in all cases 2.7N. The amount of the zirconyl-alizarin sulfonate mixture used for each is given below -

No. 14 - 70 ml
No. 15 - 60 ml
No. 16 - 65 ml
No. 17 - 75 ml
No. 19 - 85 ml

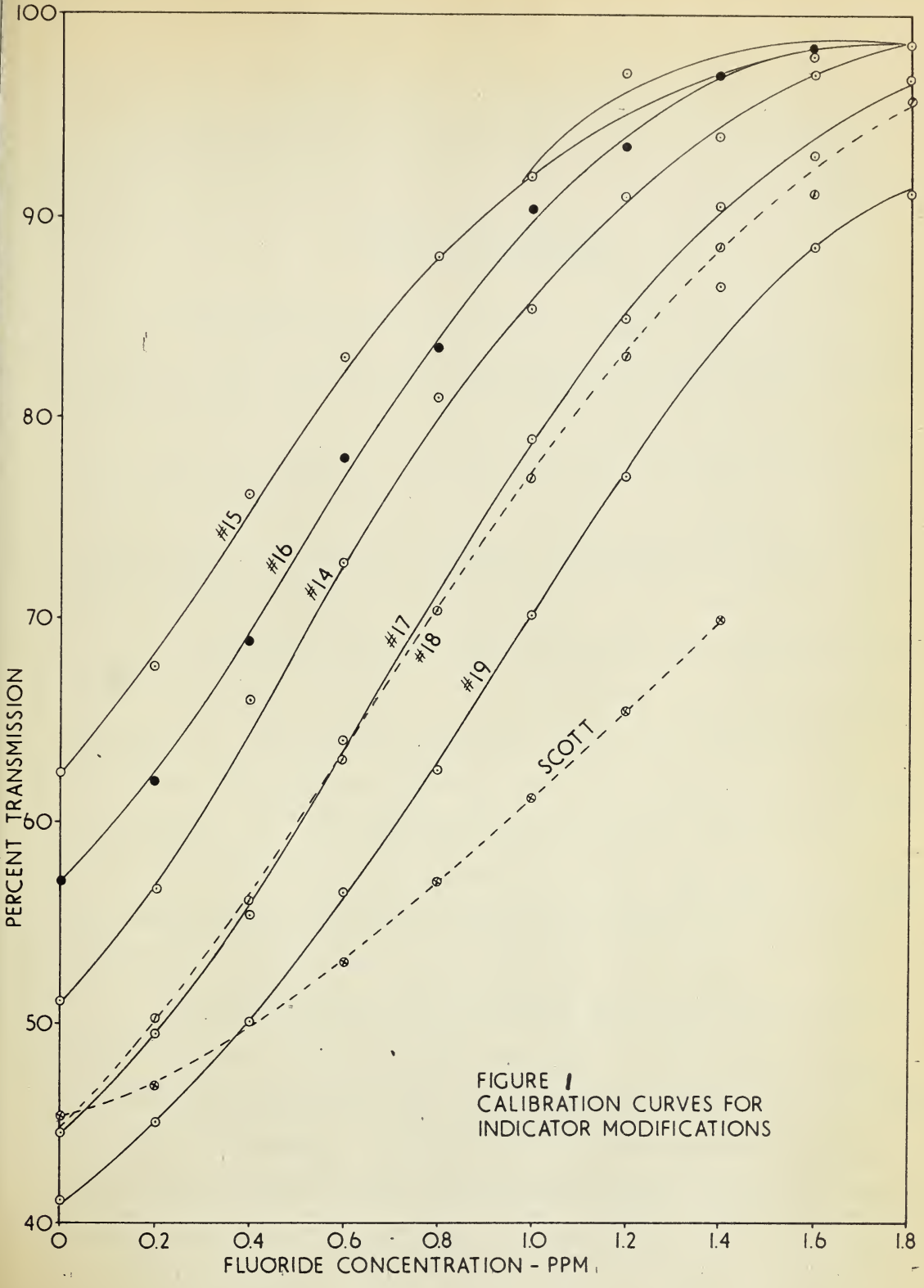


FIGURE 1
CALIBRATION CURVES FOR
INDICATOR MODIFICATIONS

PART 2 - DISTILLATION

The distillation step was the one in which the major interest, and difficulty, lay. Previous work in this laboratory on the proposed modification of the Thrun (10) procedure led to inconclusive results and the desired results were not obtained. It had been hoped that it would be possible to develop a distillation technique less tedious and more amenable to routine analytical procedures than those existing. An accuracy of $\pm$ 0.1 ppm was desired as being sufficient for many purposes.

The purpose of this the distillation is to remove the fluoride from interfering species. Two types of interferences are reported by Megregian and Solet (5). The first type retains the fluorine in a form refractory to distillation; the second type interferes with the indicator in the subsequent determination. Al^{+++} and silica are of the first type. Of the second type the most important are the volatile ones such as Cl^-, $PO_4^{\equiv}$, $SO_4^{\equiv}$, NO_2^-, and NO_3^-. These interfere either through the introduction of excess H+ or by the complexing effect they may have with the indicator.

Willard and Winter (16) in a classic paper in the field report on several methods of fluoride analysis. The method of determination involves titration with thorium nitrate and the distillation technique used varies with the nature of the sample. Basically the fluoride is steam distilled from either perchloric acid, or sulfuric acid, hydrogen fluoride, fluosilicic acid, or fluoboric acid.

The Methods of Analysis of the AOAC (3) give detailed analytical procedures for the determination of fluoride in a variety of substances of agricultural interest. These procedures, whether titrimetric or colorimetric, are uniformly long and tedious. In general the distillation steps are modifications of those reported by Willard and Winter (16). These may involve a double distillation, first from sulfuric acid and then from perchloric acid or a single distillation from either. Depending upon the technique the distillation may require over an hour and involve distillate volumes in excess of 400 ml.

For the analysis of water samples Thrun employed á straight distillation from sulfuric acid with a stream of air being passed through the boiling mixture to reduce bumping but mainly to assist in the volatilization of the fluoride. His subsequent method of determination was based on the bleaching effect of fluoride on the aluminum lake of eriochromecyanine. This indicator shows a remarkable tolerance to interferring species and the distillation was resorted to only in extreme cases. Silver sulfate was added to assist in the retention of the chloride although other workers (5) indicate some debatability regarding the effect chloride does have especially with reference to other indicators. Thrun reports that the rate of distillation and the flow rate of air have a large effect on the degree of recovery. The faster the better. The reported optimum time of distillation of 90 ml is 14 minutes.

Our method was a modification of the Thrun procedure in which the

passage of air is omitted, bumping being controlled by the presence of a few glass beads. The method briefly is as follows: 110ml of water containing the fluoride to be distilled plus a few glass beads were placed in a 500 ml round-bottom flask, 40 ml of concentrated sulfuric acid is carefully layered under this. The elbow tube, condenser, and adapter are then connected. The apparatus was all glass with a condenser length of 51 centimeters. The Meker burner was then turned on full and the apparatus shaken. Following Thrun, 90 ml of distillate were collected, this being the optimum volume for completeness of recovery while keeping the sulfate concentration in the distillate as low as possible. (This and other factors will be discussed later). The distillate was made up to 100 ml, a 25 ml aliquot was taken for the analysis for sulfate and the remaining 75 ml again made up to 100 ml and analyzed colorimetrically for fluoride. A diagram for the apparatus is given in Figure 2.

The analysis for sulfate used was the tetrahydroquinone titration. This the addition to the sample to be analyzed of 25 ml of ethyl alcohol, the THQ indicator and a smidgen of silver nitrate to sharpen the end-point. This is then titrated with a standard solution of barium chloride. The extent of the effect of sulfate concentration on the indicator had been studied and reported on by Walker et al (11). Their figures were used throughout this work. In spite of the large damping effect which the sulfuric acid of the indicator has, it had been found that sulfate in excess of 150 ppm required the application of a correction term. The correction terms of Walker et al are reprinted in Table 2.

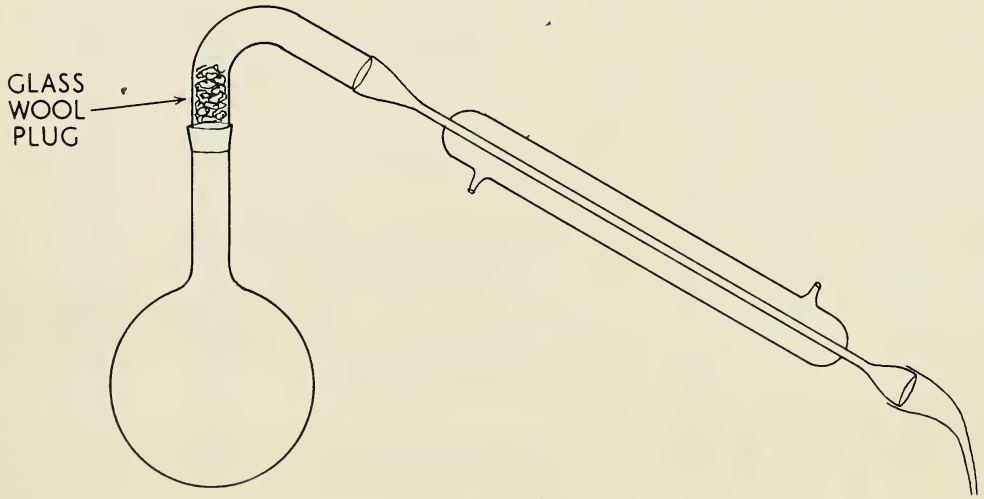


FIGURE 2
DISTILLING APPARATUS SHOWING POSITION OF GLASS WOOL PLUG.

TABLE 2

EFFECTS OF SULFATES ON DETERMINATION OF FLUORIDES

F^- Present ppm.	ppm. SO_4 Added: 200	600	1000	1600	2400	2800	3200
	F^- Found ppm.						
0.0	0.1	0.2	0.3	0.5	0.6	0.7	0.8
0.2	0.3	0.4	0.5	0.7	0.8	0.9	1.0
0.4	0.5	0.6	0.7	0.9	1.0	1.1	1.2
0.6	0.7	0.8	0.9	1.1	1.2	1.3	1.4
0.8	0.9	1.0	1.1	1.3	1.4	1.5	
1.0	1.1	1.2	1.3	1.5			
1.2	1.3	1.4	1.5				
1.4	1.5						

It was found that the sulfate concentrate of the distillate could be reduced from values averaging roughly 1000 ppm. to ones of the order of 200 ppm by placing a plug of glass wool in the vertical section of the elbow just below the bend. This enables us to increase the volume of distillate to 100 ml thereby improving the chances of complete recovery.

In Table 3 the effect of volume on sulfate concentration in the distillate and also the marked reduction possible in the presence of the glass wool plug is shown.

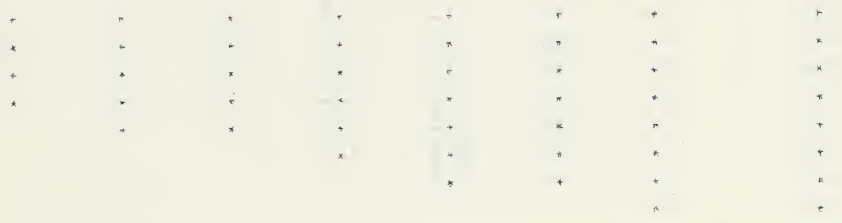


TABLE 3

EFFECT OF VOLUME ON $SO_4^=$ CONCENTRATION IN THE DISTILLATE

Volume of Distillate, ml.	$SO_4^=$ Concentration Without Glass Wool, ppm.	$SO_4^=$ Concentration With Glass Wool ppm.
60	270	120
60	220	
70	300	110
70	440	
80	550	170
80	450	
90	1,350	140
90	1,180	

It would appear that most of the sulfate distills over during the last 10 ml. of distillation. The glass wool plug would tend to reduce the sulfate carried over by mechanical action and also that which distills over since it would act as a short reflux column.

Table 4 gives the sulfate concentration and the time of distillation for a distillation series in which 100 ml. of distillate were collected. This is representative of the values encountered during the work on this phase of the project and these data will be left out of subsequent tables. At this time no reference will be made to the fluoride recovery.

TABLE 4

$SO_4^=$ CONCENTRATION AND TIME OF DISTILLATION

Sample No.	$SO_4^=$ Concentration in ppm.	Time of Distillation Minutes and Seconds
1	310	21:39
2	450	20:52
3	190	16:23
4	310	17:56
5	410	22:55
6	400	17:35
7	290	15:39
8	390	17:55
9	290	19:27
10	370	14:50
11	220	14:42
12	270	16:58
13	250	17:19
14	250	16:08
15	190	13:40
16	360	14:58
17	190	15:39
18	350	15:53
19	220	12:10
20	430	14:30

The sulfate concentration varies over quite a range. No direct relation between time of distillation and sulfate concentration in the distillate is apparent. (Nor did time of distillation seem to influence the recovery of fluoride.) There was however a relationship between the packing of the glass wool plug and the sulfate concentration in the

distillate and also the length of time which any one plug was used. The less tight the packing and the longer a plug was used the higher the sulfate concentration of the distillate. The plugs were changed after every second series of distillations. Generally a series consisted of 20 samples and since 4 units of distillation apparatus were used throughout the work, each plug was used approximately 10 times.

Since it has been generally agreed (4, 9, 16) that a temperature of distillation in excess of 150°C is necessary for complete recovery, a study was made to determine how our distillation technique fulfilled this condition. This is the reason for the use of a steam distillation technique in which water is added as it is removed so that the acid concentration will remain at a level high enough to result in the required temperature. Table 5 contains the temperature of distillation as a function of volume of distillate. 110 ml. of water and 40 ml. of concentrated sulfuric acid were used as the starting mixture.

It can be seen from the table that the last 30 ml. of distillate come over at a temperature at and above that recommended. Hence we felt that the condition of temperature, at least, was adequately fulfilled.

In order to determine the effect of volume of distillate on the recovery of fluoride a number of series of distillations were carried out in which the volume of distillate collected was varied from 80-100 ml.

TABLE 5

TEMPERATURE OF DISTILLATION

Volume of Distillate, ml.	Time	% H_2SO_4, w/v	Temperature, °C
0	0	37.3	111.0
10	1:30	39.2	113.5
20	2:58	41.3	116.0
30	4:30	44.4	119.0
40	6:05	47.5	123.0
50	7:48	51.2	128.5
60	9:40	55.5	136.5
70	12:00	60.3	148.0
80	14:32	66.0	165.0
90	17:34	73.2	192.0
100	21:40	81.9	238.0
110	26:55	93.2	303.0

Table 6 gives the fluoride found and the fluoride actually added for concentrations from 0-1.8 ppm. The values under fluoride are corrected for sulfate concentration and for the volumetric manipulations. All work was done in duplicate.

Figure 3 gives the fluoride found versus fluoride added for these series of distillations with the straight line that represents complete recovery without error. The numbers on the graph indicate the occurrences of each value. The broken lines indicate the desired limits of accuracy.

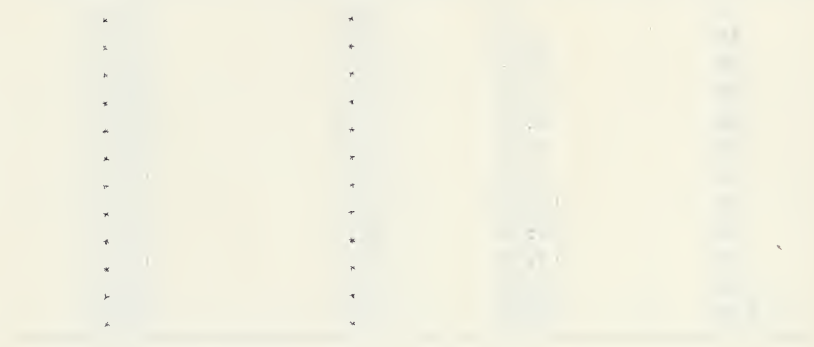


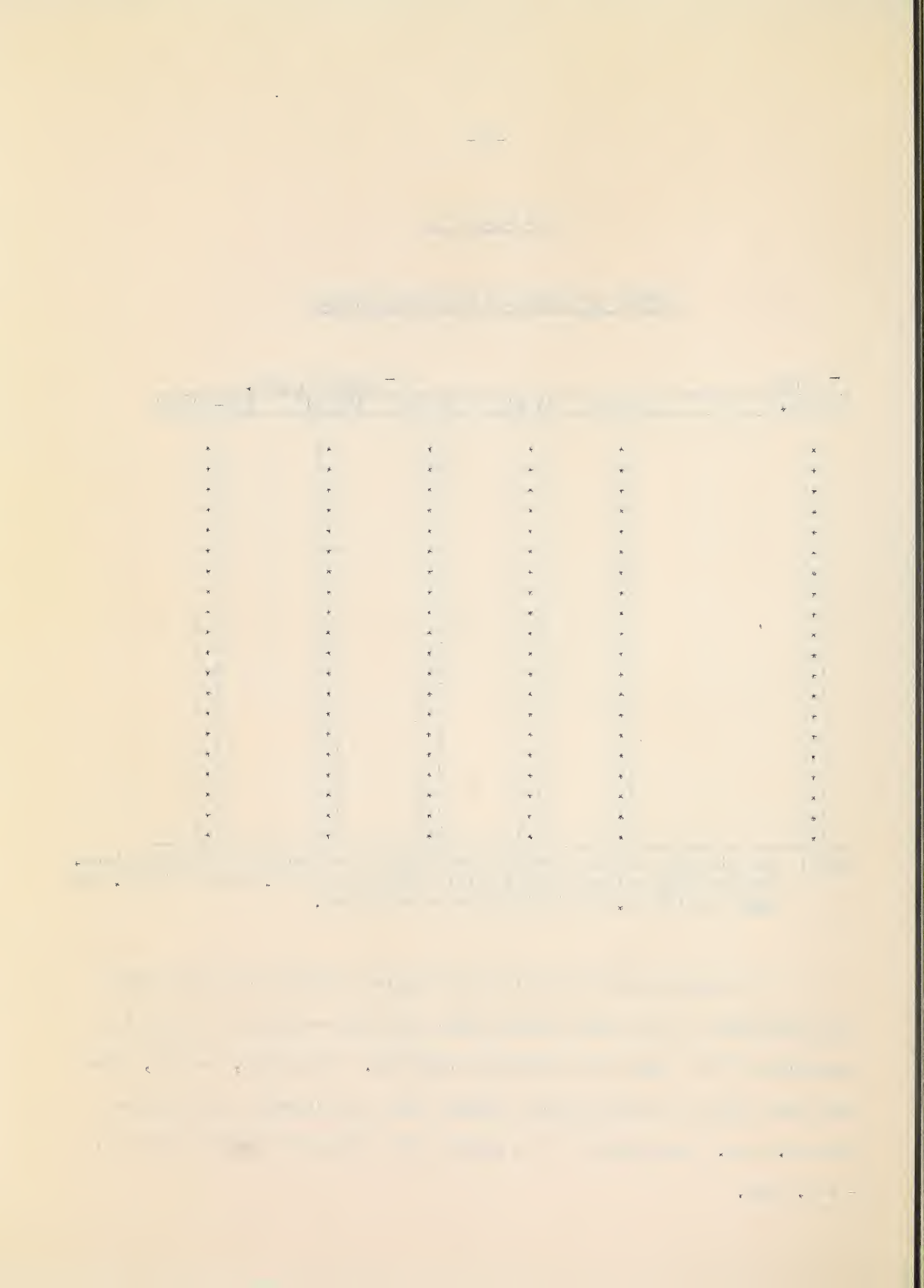
TABLE 6

EFFECT OF VOLUME ON FLUORIDE RECOVERY

F^- added in ppm.	F^- found in ppm.				
	80 ml	85 ml*	90 ml	100 ml*	100-105 ml
0.0	0.3	0.3	0.2	0.1	0.2
0.0	0.4	0.4	0.1	0.1	0.2
0.2	0.4	0.4	0.4	0.3	0.4
0.2	0.6	0.8	0.4	0.3	0.4
0.4	0.5	0.6	0.4	0.4	0.4
0.4	0.5	0.6	0.3	0.5	0.4
0.6	0.6	0.7	0.7	0.5	0.6
0.6	0.7	0.9	0.8	0.5	0.6
0.8	0.7	0.8	0.7	0.7	0.9
0.8	0.7	0.9	0.6	0.9	0.9
1.0	0.8	1.6	1.1	0.9	1.1
1.0	0.9	1.2	1.5	1.0	1.0
1.2	0.9	1.3	1.0	1.0	1.2
1.2	1.0	1.3	1.1	1.3	1.1
1.4	1.4	1.5	1.5	1.3	1.2
1.4	1.6	1.5	1.5	1.3	1.4
1.6	1.4	1.8	1.4	1.6	1.6
1.6	1.4	1.8	1.2	1.8	1.8
1.8	1.8	1.6	1.8	1.9	1.7
1.8	2.1	2.1	2.0	2.0	1.7

NOTE: The starred columns represent averages of two series of distillations. For the last column a total water volume of 105 ml. rather 110 ml. was used and 100 ml. of distillate were collected.

As can be seen from Table 6 and Figure 3 little can be said about the efficiency of this distillation except that the recovery is inconsistent regardless of the volume of distillate collected. In general, however, these and other series of distillations indicate that for fluoride concentrations above 0.4 ppm. the majority of the results fall within the desired limits of + - 0.1 ppm.



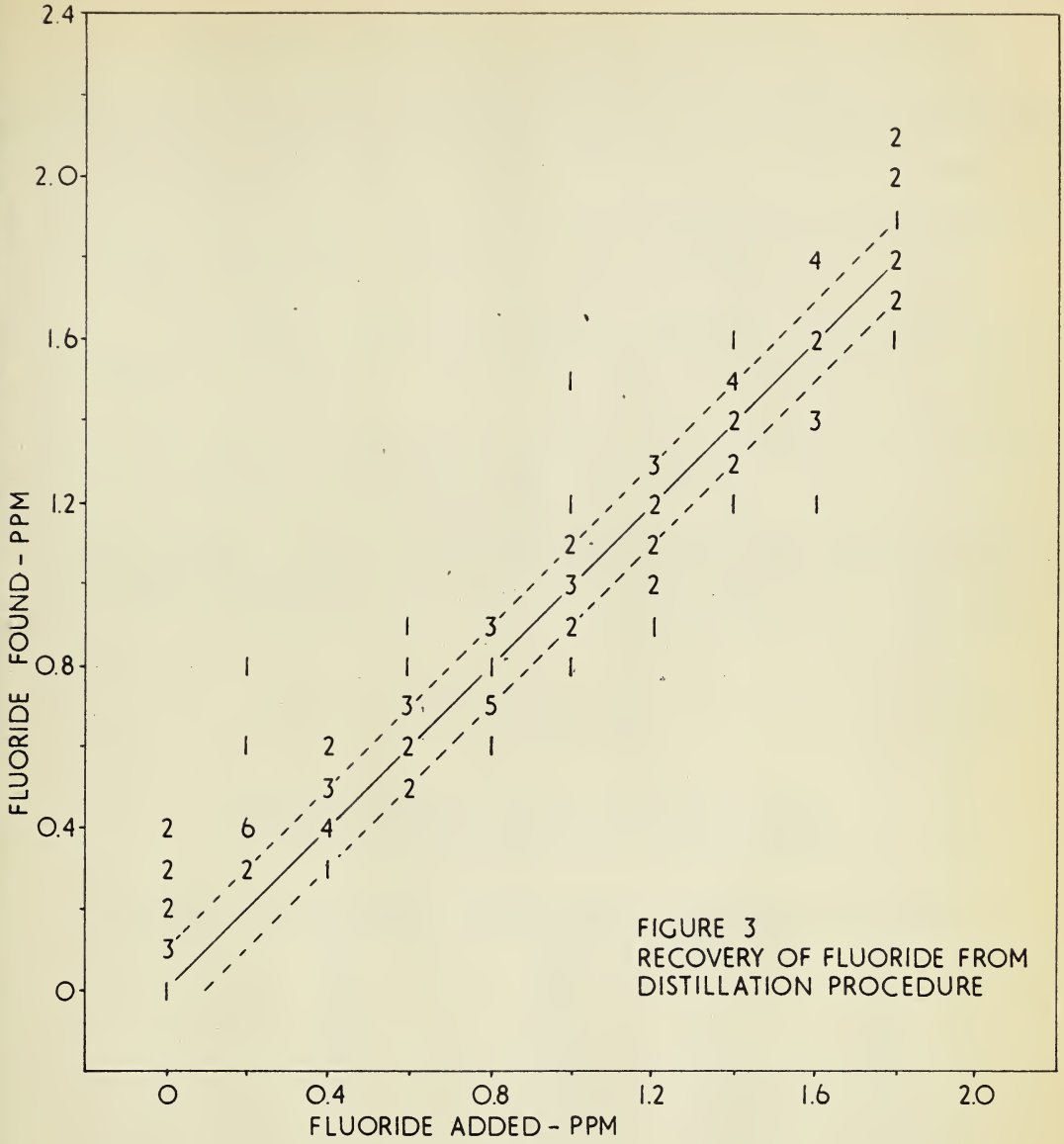


FIGURE 3
RECOVERY OF FLUORIDE FROM
DISTILLATION PROCEDURE

The overall nature of the results would indicate that there is a high positive blank with incomplete recovery. The source of this positive error is not readily ascertained. One possibility is the sulfuric acid used as can be seen in Table 7; one other was mentioned recently in a conversation with Dr. Harris, namely that fluoride was being extracted from the glass.

The data of Table 7 was obtained in series of distillations carried out first in an attempt to discover the amount of acid actually required to volatilize all of the fluoride and secondly to investigate the possibility that the acid or water was introducing the positive blank. In this series varying amounts of water and acid containing 0 ppm. and 0.6 ppm. fluoride were distilled and 90 ml. of distillate collected. The sulfate correction is not shown.

TABLE 7

EFFECT OF VOLUME OF ACID ON FLUORIDE RECOVERY

Sample	Volume H_2SO_4	Volume H_2O	F^- added	F^- found
1	10	140	0	0.1
2	20	130	0	0.2
3	30	120	0	0.3
4	40	110	0	0.3
5	10	110	0	0.1
6	20	110	0	0.2
7	30	110	0	0.3
8	40	110	0	0.3
9	10	140	0.6	0.3
10	20	130	0.6	0.4
11	30	120	0.6	0.5
12	40	110	0.6	0.7
13	10	110	0.6	0.4
14	20	110	0.6	0.6
15	30	110	0.6	0.7
16	40	110	0.6	0.7

Since the distillation was usable over the range of 0.4-1.8 ppm fluoride, it was decided to proceed to the fusion step.

PART 3 - FUSION

The fusion step is necessary for samples containing a large amount of organic materials and for samples which are difficult to decompose and contain the fluoride in a refractory form. Since hydrogen fluoride is volatile, basic fluxes must be employed. The Methods of Analysis of the AOAC (3) uses a suspension of calcium oxide extensively and for extremely refractory materials such as cryolite employs a flux consisting of equal weights of anhydrous potassium carbonate and sodium carbonate. Previous work in this laboratory (12) investigated a number of fusion materials, viz.: sodium hydroxide, potassium hydroxide, sodium peroxide, barium peroxide, magnesium nitrate and calcium hydroxide.

Magnesium nitrate was found unsatisfactory possibly due to the volatilization of the fluoride, since the salt is acidic. The calcium and barium compounds also gave erratic results. Here the error probably arose from the formation of insoluble compounds. During our work on this phase of the project sodium hydroxide was not studied since sodium fluoride decomposes at 384°C, a temperature below that at which the fusion was to be carried out.

In a preliminary study with starch and known amounts of fluoride it was found that the violence of reaction of sodium peroxide was so great as to constitute a disadvantage. Since it decomposes rapidly to sodium hydroxide

and water it was felt that it would offer little advantage over sodium hydroxide and its use was discontinued.

In the preliminary studies of the fusion step, the method followed was this: known amounts of fluoride, 0.5 gm. of starch and the fusion material were placed in a nickel crucible; the volume was made up to 20 ml. with water and mixed. The sample was evaporated to dryness and fused for one hour at approximately 650-700°C. in an electric muffle furnace. The fusion residue was leached with hot water, made up to 110 ml. and distilled and analyzed as before.

In this procedure the greatest source of mechanical error lay in the evaporation step since spitting was very hard to control. It was later found that good results could be obtained by the elimination of the addition of water, that is, by using a dry fusion mixture of the organic material and the flux.

Table 8 illustrates some of the results obtained. The values in the last column are corrected for sulfate carried over in the distillate. These results are the average of 2 series carried out with potassium hydroxide as the flux.

In this work samples containing 0.00 mg. and 0.02 mg. were distilled only as a blank, since this was the region of greatest error in the distillation step. It can be seen from the data of Table 8 that the fusion step introduces no new error above that of the distillation itself.

It was found that the temperature of fusion could be raised to 800°C. without introducing any new error. At this temperature, however, the molten

potassium hydroxide attacked the nickel crucible, thus later fusions were carried out at approximately 700°C.

TABLE 8

FUSION WITH KOH

F^- added, mg.	F^- found, mg.
0.00	0.04, 0.03
0.02	0.05, 0.05
0.04	0.07, 0.06
0.06	0.08, 0.07
0.08	0.08, 0.10
0.10	0.09, 0.10
0.12	0.10, 0.10
0.14	0.13, 0.13
0.16	0.15, 0.17
0.18	0.17, 0.19
0.00*	0.03, 0.03
0.02*	0.04, 0.05

NOTE: starred values are for distillation step only.

PART 4 - FUSION AND ANALYSIS OF TOOTH MATERIAL

A number of teeth had been ground up and mixed so as to assure a uniform sample. Portions of this material were studied with a view towards investigating the recovery of the fusion with more refractory materials. In this work the fusion of potassium carbonate and sodium carbonate was also studied. Table 9 gives the results of this work.

TABLE 9

FUSION OF TOOTH MATERIAL WITH KOH AND WITH K_2CO_3-Na_2CO_3

Fusion Material	F^- found, mg.	% Fluoride
KOH	0.13	2.6 x10-2
KOH	0.12	2.2
KOH	0.10	2.0
KOH	0.15	3.0
KOH	0.17	3.4
KOH	0.16	3.2
K_2CO_3-Na_2CO_3	0.16	3.2
K_2CO_3-Na_2CO_3	0.16	3.2
K_2CO_3-Na_2CO_3	0.16	3.2

There is little to choose between the two fusion materials. Since the carbonate mixture, however, produces copious quantities of CO_2 upon neutrilization, its use was discontinued. It has also the added disadvantage

of requiring a correction of 0.01 mg. fluoride per gram of flux as determined experimentally. Potassium hydroxide requires no correction.

In order to further investigate the applicability of the fusion step, a number of Eskimo teeth were analyzed following the procedures outlined in parts 1, 2, and 3.

The teeth were supplied to us by Dr. Carey, Dental Health Officer of the Charles Camsell Indian Hospital. The teeth were extracted from 20 adult Eskimos of the Cambridge Bay area and information was supplied as to the patients age, sex and type of diet and whether the particular tooth was carious.

Each tooth was given the following treatment: It was soaked a day in acetone, then in ethyl alcohol, followed by treatment for similar periods in two portions of water. The tooth was then dried for two days in an oven at 110°C. After weighing, the tooth was ground to fine powder in a mortar and pestle. Because of the hardness and brittleness of the tooth, it was placed in a copper cylinder on the mortar and broken with the small end of the pestle to prevent loss of sample by pieces flying out.

The next step was to take a mixture of the tooth with 3 gm. of potassium hydroxide for each 0.5 gm. of tooth weight in a nickel crucible and fuse for one hour in an electric furnace at 650-700°C. After cooling, the melt was treated with hot water and brought up to a specific volume depending upon the weight of the tooth. Referring to Table 9 the volumes used for teeth Nos. 1, 3, 4, 5, 6, 11, 13, 16, 18, and 19 was 200 ml., for

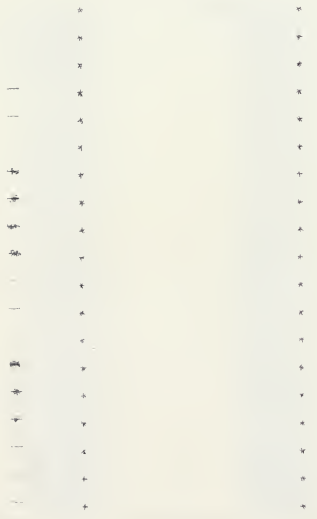
teeth 9 and 12 was 250 ml., for 7 and 17 was 300 ml., for 8 and 10, 500 ml., and for 2 and 14, 600 ml.

Duplicate 100 ml. aliquots plus 40 ml. of concentrated sulfuric acid and 10 ml. of water were taken and analyzed as indicated before. An average of the two determinations (which agree very well in all cases) was made and the total fluoride content of each tooth calculated. Results are shown in Table 10. One tooth was lost through spillage. Column 2 indicates the general condition of the teeth of the patient.

TABLE 10

ANALYSIS OF ESKIMO TEETH FOR FLUORIDE

Tooth No.	Caries Incidence	Weight of Tooth	% Fluorine
1	-	0.8624 gm	0.067 -
2	±	2.8370	0.034 ±
3	+	0.6379	0.088 +
4	-	0.7511	0.043 -
5	-	0.9570	0.034 -
6	-	1.0805	0.024 -
7	+	1.3860	0.063 +
8	+	2.1637	0.046 +
9	+	1.0816	0.043 +
10	+	2.0498	0.037 +
11	-	0.8044	0.054 -
12	-	1.2778	0.038 -
13	-	0.7810	0.074 -
14	±	2.6532	0.025 ±
15	±	1.0105	0.050 ±
16	±	0.9058	0.045 ±
17	-	1.3634	0.040 -
18	-	0.6808	0.062 -
19	-	0.7946	0.066 -



The percentages of fluoride obtained are somewhat larger than have been reported by some analysts but are of the same order as those of Armstrong for teeth from high fluoride areas (2). The diet of fish and seal with some consumption of the bones by the Eskimoes could account for this.

There is a distinct variation of fluoride content with the size of the tooth, ie. with the type of tooth it is, cuspid, bicuspid, or otherwise. This information was, unfortunately, not supplied. The occurrence of caries among Eskimoes seems to be a function of the extent to which their diet has been influenced by the white man.

SUMMARY

It can be concluded that the methods outlined in the experimental sections can be applied to the analysis for fluorine of organic materials over a limited range with moderate accuracy. The Modified Scott indicator is rapid, accurate and amenable to routine colorimetric procedures. The fusion with potassium hydroxide introduces no error into the analysis. There is, of course, no definite proof that the fusion releases all of the fluoride. However, since the carbonate flux is reported to be sufficient for highly refractory compounds and the potassium hydroxide gave results in good agreement with this flux, it would seem that the method can be relied upon.

The distillation procedure was moderately accurate over the range of 0.6 to 1.8 ppm. The results indicate, however, that recovery is incomplete with a large positive error arising from some unknown source. We believe that it can be concluded that a simple distillation of 100 ml. is not sufficient for the total recovery of fluoride and that either a steam distillation or some other assistance for complete volatilization is required, such as, for example, Thrun's use of a stream of air.

The source of the positive blank cannot be definitely decided upon. Two possibilities present themselves, viz: the presence of fluoride contaminant in one of the reagents used, either the water or the sulfuric acid, or alternately, the extraction of the fluoride from the glass of the apparatus.

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SECTION II

THE THERMODYNAMICS OF THE WOOL KERATIN-WATER SYSTEM

INTRODUCTION

A considerable liberation of heat is observed when water is adsorbed on dry wool keratin. The heat of wetting and, from it, the net integral heat of adsorption may be obtained experimentally through the use of calorimetric techniques. The differential heat of adsorption, $\overline{\Delta H}$, is of great theoretical significance since this gives the partial molal heat at every water content over the range studied.

Hedges(13) measured the heat of wetting of wool over the range of 0-18% water content. His method employed a Dewar flask and a Beckman thermometer with subsequent electrical duplication of the temperature rise. This method did not permit thermal measurements for samples with a water content greater than 18%.

Alternatively the differential heat of adsorption may be determined from calculations based on isotherms at two different temperatures with the Clausius-Clapeyron equation $\Delta H = \frac{RT_1 T_2}{T_2 - T_1} \frac{\ln X_1}{X_2}$

where X_1 and X_2 - the relative vapor pressures at the two temperatures, T_1 and T_2.

This was done by Bull (5) on isotherms he determined for a number of proteins. Bull used the Gibbs-Helmholz equation. As pointed out by Dole and McLaren (9) this is incorrect. They recalculated Bull's data (5) using the above equation. Since this is only an indirect calculation based on the two isotherms, the results are uncertain at low and at high ranges of water contents. These, however, are the more important parts.

The agreements between the two methods are good except at the upper range of water contents. However, the values extrapolated to high water contents from Hedge's data indicate that $-\Delta H$ increases at high water

contents. The use of incomplete data led Cassic (7) to conclude that heat is adsorbed and that the differential entropy change becomes positive at large values of n. Davis and McLaren (8) point out that this positive entropy change is observed with soluble proteins but does not occur with insoluble fibrous proteins.

Previous work in this laboratory by Dunford and Morrison (10, 11,12) on silk fibroin has shown that the upswing in $-\overline{\Delta H}$ and $-\overline{\Delta S}$ at high water contents given by Davis and McLaren (8) does not occur. All of the undulations in the $-\overline{\Delta S}$ curve except the major maxima and minima are ascribed rightly to experimental error by Davis and McLaren. However, they make no attempt to explain the occurrence of these maxima and minima.

The purpose of this work was to determine experimentally the heats of wetting over the entire water content range from 0 to saturation for samples with various amounts of adsorbed and desorbed water. These values plus free energy changes calculated from the sorption isotherm would lead to values of the thermodynamic properties for the system over the entire water content range.

Some Chemical and Physical Properties of Wool Keratin

Most of the following facts were obtained from "Wool - Its Chemistry and Physics" by Alexander and Hudson (1).

Wool keratin is considered to consist, chemically, of 18 different amino acids. Of these, 5, arginine, cystine, glutamic acid, leucine, and serine make up 58% by weight of the total. These basic components are mainly arranged in a long chain with a molecular weight of approximately 80,000 with about 800 residues per molecule.

The non-protein portion of cleaned, natural wool represents a maximum of 1% of the total. Thus its chemical and physical properties can

be considered to be largely based on the protein portion.

From 50 - 90% of the gross structure is considered to be amorphous. However, the chains themselves appear in two well defined forms: α keratin which is the natural form and β keratin which is the structure of stretched wool.

The subsequent manner in which these chains agglomerate to form the wool fibre is open to some debate. However, in general it can be said that the molecules are cross-linked with hydrogen bonds and a few terminal disulfide bonds to form subfibrils, which in turn interlock to form the fibrils. The lenghwise arrangement of these fibrils comprise the cortex of which 90% of the wool fibre consists.

SAMPLE PREPARATION AND DETERMINATION OF THE ISOTHERM

Wool keratin was obtained from Dr. P. Larose, National Research Council in the form of 60's quality yarn which had been extracted with alcohol and ether and finally equilibrated to a pH of 5.0.

A 1 to 2 gram sample was placed in the lower compartment of a glass reaction cell illustrated in Fig. 1c. The upper and lower compartments of the cell are separated by a glass septum which is broken at the beginning of a calorimetric experiment. At that time the upper compartment contains distilled water and the lower compartment contains the evacuated keratin sample.

Each sample is evacuated at 80°C (Bright et al. (4)) for at least 6 hours. Water vapor is then introduced by means of the same apparatus as was used for the determination of the sorption isotherm.

For samples with desorbed water the samples were first saturated with water vapor to at least 30% water content. This usually required 2-3 days. Then enough water was removed by pumping to give a sample with the desired amount of desorbed water.

A detailed procedure of sample preparation is as follows:

1.) Weigh A (empty) and B parts (Fig. 1b) together in air.

2.) Add 1-2 gm wool keratin to the lower compartment of A, place a weighed loose glass wool plug near the open end to prevent burning.

3.) Join A and B in a flame. Evacuate through the stopcock.

4.) Weigh the assembly A plus B after proper evacuation.

5.) When required, admit water vapor (adsorbed or desorbed) and weigh.

6.) Remove B from A by sealing off A with a flame.

7.) Attach tube C, add distilled water to the upper compartment of A, place the loop of wire over the glass septum and instal in the calorimeter.

The glass cell is made from Pyrex tubing of 28 mm. and 10 mm O.D. A new cell is made for each sample.

Water Vapor Sorption Isotherm

The water vapor adsorption and desorption isotherms on wool keratin (8.200 gm) were measured at 24.7°C with a vacuum apparatus like that used by Wiig and Juhola (21) for charcoal. Their dibutyl phthalate manometers were replaced by Apiezon Oil B Manometers. The stopcocks were greased with Dow Corning Silicone High Vacuum Grease which behaved better than Apiezon L grease. The keratin was evacuated at 80°C for 12 hours. Then at least 24 hours was allowed for equilibration for each point on the isotherm.

The results of these measurements are given in Table 3 and Fig. 4.

APPARATUS

The stationary adiabatic calorimeter used in this work is a modification of one described by Howard and Culbertson (14). The major modification lay in the use of water to surround the outer calorimeter jacket, instead of an evacuated space equipped with aluminium radiation foils; also thermistors were used to measure the temperature change rather than thermocouples.

The calorimeter and accessories were largely constructed by D. E. Thyne. (For details of dimensions reference should be made to Appendix 1).

The calorimeter and jacket are shown in Figure 1a. The calorimeter proper (A) is a nickel plated cylinder, 5.5 inches by 2 inches, prepared by the Machine Shop and supported on a lucite pedestal (E). (B), is the calibration heater. It consists of manganin wire non-inductively wound on a lucite frame with a resistance of approximately 100 ohms. The sample cell (C) is placed as shown with a loop (D) around the glass hook. Adiabatic control is sensed by a thermel consisting of 42 copper-constantan junctions (F) wound on a lucite frame. The conducting medium (G) was vacuum pump oil.

The outer brass jacket (H) was also made by the machine shop. The flanged jacket lid (I) was fitted with gasket material and greased periodically with Varniton stopcock grease to ensure a water tight seal. On this lid was fitted a brass tube, (J) to act as a bearing for the sample cell. This was fitted with a length of rubber tubing in which was placed a short piece of corkborer, projecting above the water level. The side arm (K) which also projected above the water, carried the electrical leads for the thermocouples and for the heater. After the wires had been put in place this was filled with paraffin.

The upper end of the sample tube was connected to an oscillatory

device which rotated the sample cell back and forth through about 130° in order to hasten the thermal equilibration of the calorimeter and oil. This device was constructed from a geared D.C. motor and a few Meccano parts. Each stroke required about 1.5 seconds. No heating effect arising from this rotation could be detected. The time lag for transference of heat uniformly from the cell (or from the heater) to walls of the calorimeter as detected by the thermal was between 100 and 150 seconds.

The calorimeter and jacket assembly was supported in the water bath by a specially prepared tripod. The water bath consisted of a large crock in a wooden box packed with insulating material. During a run the water level was maintained at about 1 inch above the lid of the jacket. The bath was stirred by four rapidly rotating stirrers which transferred the last of any added hot water to the thermocouples in about 3 seconds. The temperature of the bath was maintained at the same temperature as the calorimeter with streams of hot and cold water added through stopcocks mounted of the wall of the box. The bath was covered with a lucite lid through which had been bored holes for the various pieces of apparatus. In the centre of this lid a lucite disk about 5 inches in diameter covered the central opening through which samples were changed.

During the period between samples, the bath was thermostated at 24.5°C so that the apparatus would be equilibrated at the time of a run.

The temperature rise of the bath, and hence of the calorimeter was measured by determining the resistance of a pair of thermistors mounted in series (Stantel type F, total resistance 3500 ohms). A Wheatstone bridge with arms in a 7:1 ratio and a sensitive galvanometer was used for this. Current was supplied by a 6 volt wet cell battery.

Thermal e.m.f. arising from the thermocouples was sensed by a mirror galvonometer (the same as used for the Wheatstone bridge circuit) with

a sensitivity of .07 microvolts per mm. deflection at one metre.

The potential drop across the heater was measured with a potentiometer that had been calibrated at one-fifth of the standard cell e.m.f. so that the 3 volt drop across the heater (and across the standard resistor) could be measured. Two 6 volt batteries is series were used to provide the working current.

The current for the heater was provided by a Nylab voltage stabilizer that operated through a Sola constant voltage transformer. This was operated at 6 volts for most samples and at 8 volts for those that required a larger amount of heat. A diagram of the heating circuit is shown in Figure 1c. The ballast resistor of about 100 ohms was used during the warming-up period of the Nylab instrument. The standard resistor of accurately known resistance (99.91 ohms) was used in series with the heater in order to eliminate the necessity of measuring the resistance of the heater. The potential drop across each could be determined; this along with a knowledge of the resistance of the standard resistor (this was re-calibrated at periodic intervals) was used to calculate the amount of heat put out by the heater. Time of current flow was measured with a Time-it electric stopwatch synchronized with the current switch. This heating circuit was obtained from "Physical Methods of Organic Chemistry" by Weissberger.

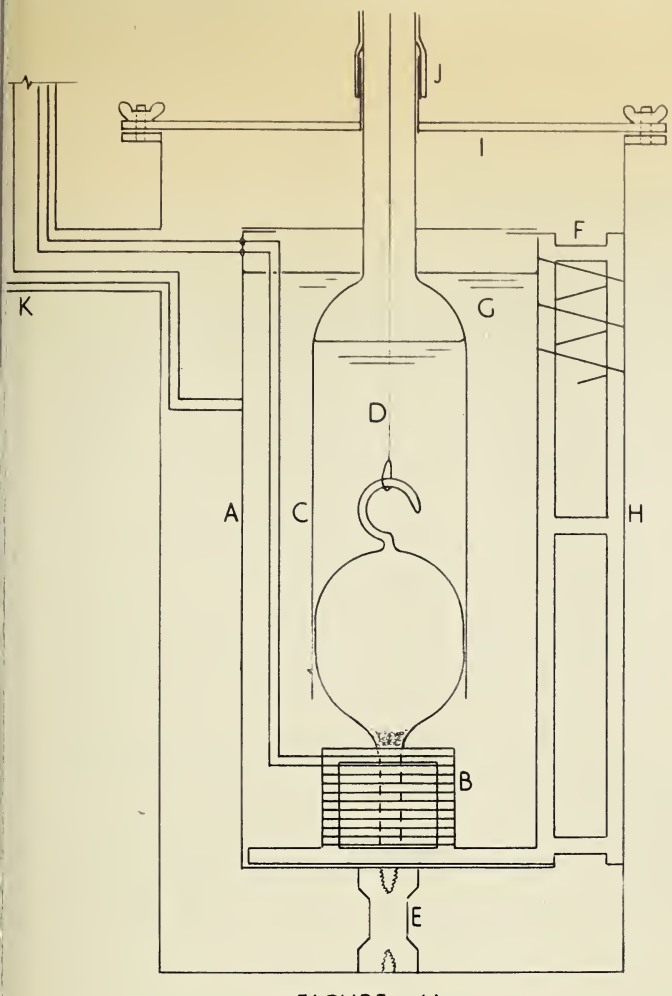


FIGURE IA

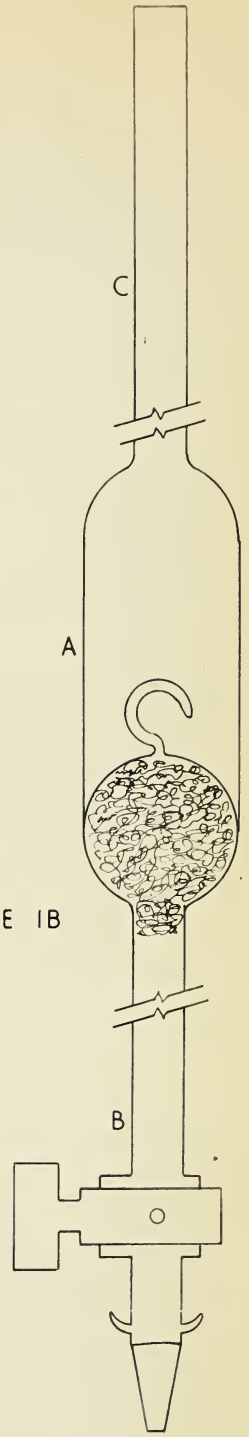


FIGURE IB

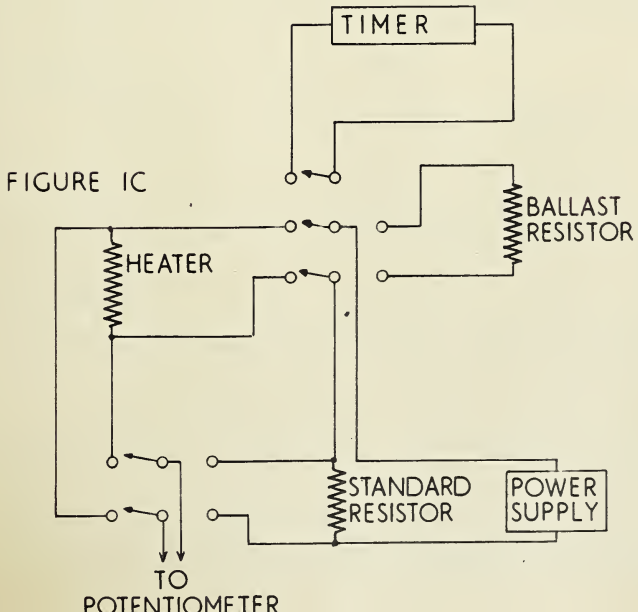


FIGURE IC

FIGURE 1
CALORIMETER, REACTION CELL
AND HEATING CIRCUIT

EXPERIMENTAL METHOD

The sample cell containing wool in the lower evacuated section and sufficient water in the upper was placed in the inner oil-filled calorimeter proper. The apparatus was thermostated at 24.5°C and allowed to equilibrate. When equilibrium had been reached (This required upwards of 12 hours, unless assisted.) the cell rotation was started and the main stirrers placed in operation. After approximately one half hour the thermostat heater was removed and shut off and the final precise equilibration made. The initial temperature of the outer bath (i.e.:and of the inner calorimeter also) was taken and the septum broken initiating the sample.

As the temperature of inner calorimeter rose, hot water was added to the outer bath maintaining equilibrium between the calorimeter and its surroundings. At five minute intervals the temperature was measured over a total time of forty minutes. This was found experimentally to be sufficient for the reaction to go to completion and equilibrium to be reached.

The heater circuit was then closed and current was passed through for a length of time sufficient to produce a heating slightly in excess of that produced by the sample itself.

During the period of heating, temperature measurements were made of the bath and also measurements of the potential drop across the standard resistor at intervals of approximately 300-500 seconds. When a sufficient temperature rise had been recorded the current was shut off and an additional 5-10 minutes were allowed before the final temperature reading was taken.

The results of this heating were plotted and the time of heating necessary to exactly duplicate the temperature rise caused by the wetting was obtained by interpolation. Since the equal temperature rises correspond to equal heat evolutions the heat of wetting was calculated using the formula:

Heat in calories $= \frac{I^2 RT}{4.183} = \frac{V_1 V_2 t}{R 4.183}$

The following is a sample run with the graphs (see Figure 2) and calculations used.

Sample D54 Room Temperature 19.3°C.

a) Wetting b) Electrical duplication

Time (secs.)	Resistance	Time (secs)	Resistance	Potential	
				Heater	Standard
0	3542.0			0.6090	0.6331
300	3522.2	0	3498.1	0.6097	0.6332
600	3509.8	600	3488.6	0.6090	0.6330
900	3503.6	1200	3473.0		
1200	3501.3	1800	3458.5		
1500	3500.2	+4	3454.4		
1800	3499.7	+9	3454.1		
2425	3498.9				

Calculations for D54 (3.34% H_2O)

t = 1730 seconds

V_1 = 0.6091 x 5 (potential drop across heater)

V_2 = 0.6331 x 5 (potential drop across standard resistor)

R = 99.91 ohms (= resistance of standard resistor)

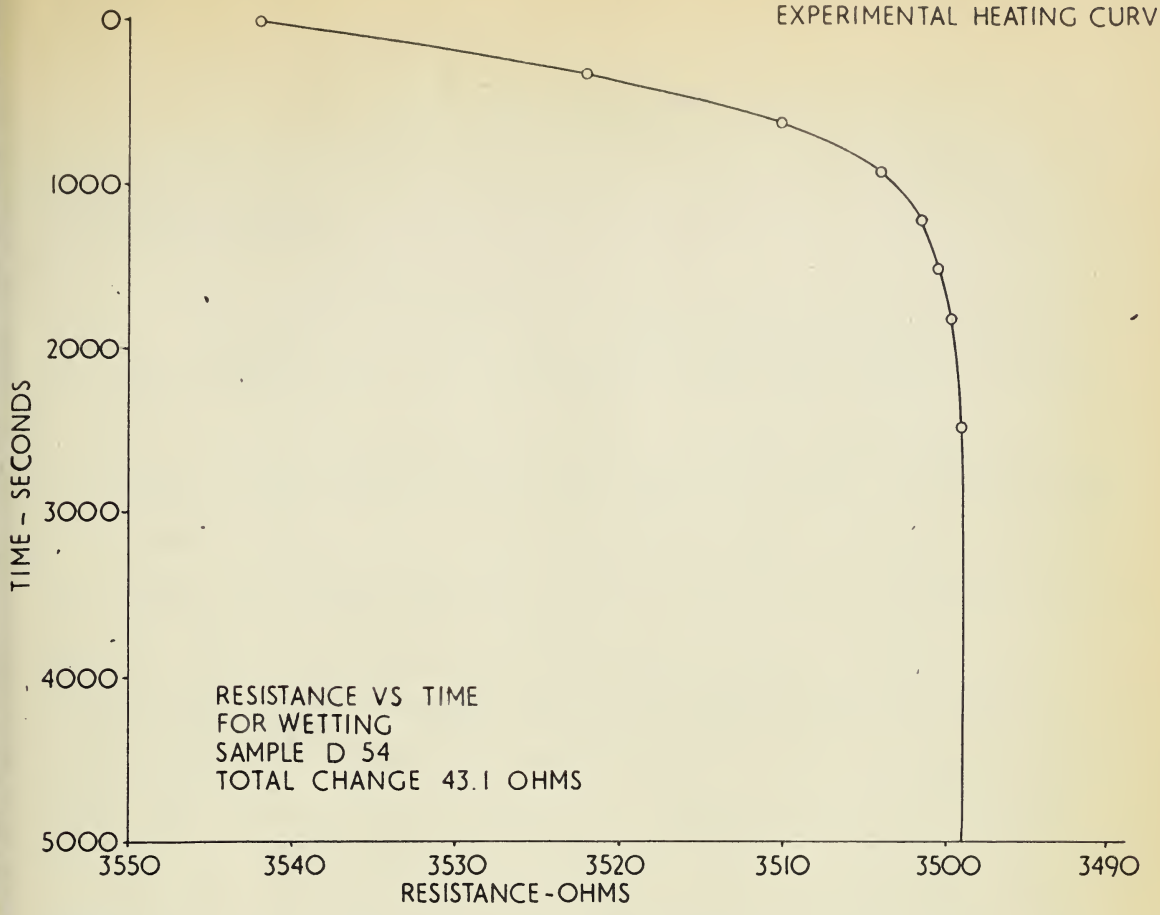
Heat = 39.9 cal.

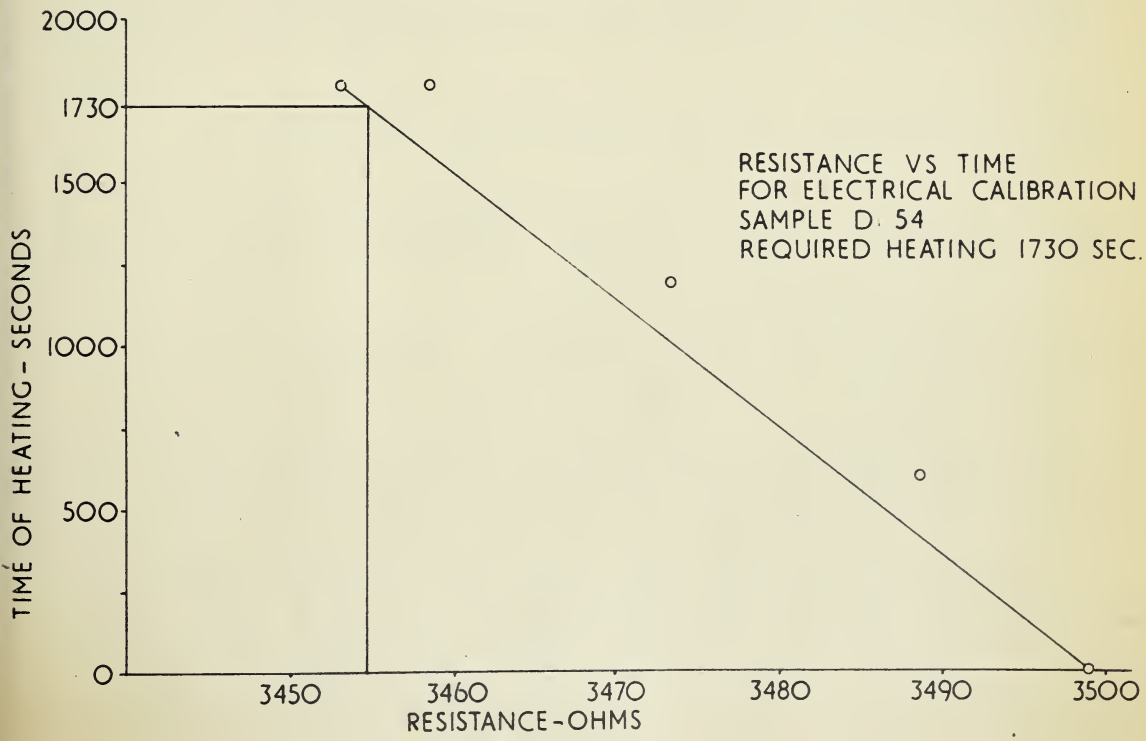
Sample weight = 2.2639 gm.

Heat of Wetting = 17.6 cal./gm.

At the end of a run the used sample was removed and the next sample installed and allowed to equilibrate at 24.5°C.

FIGURE 2
EXPERIMENTAL HEATING CURVES





RESULTS AND CALCULATIONS

Experimental Errors

The greatest source of error in the experimental determination of the heat of wetting lay in the determination of the time of heating from the graph for the electrical duplication of the temperature rise (Figure 2b). The reading of this graph was only to the nearest 5 seconds. This corresponds to a possible maximum error of 0.11 calories or, since the average weight of the samples was approximately 2 gm., an error in the heat of wetting of 0.06 cal./gm. Since any scatter due to sample preparation that appeared was outside these limits, it was felt that little would be gained by using a larger graph scale. The Wheatstone bridge could be read to 0.1 ohms $\pm$ 0.05 ohms. This corresponds to an uncertainty of approximately $\pm$ 1.2 seconds in the heating or an error of $\pm$ 0.015 calories in the heat of wetting. The sensitivity of the thermel to ambient temperature was 0.0002°C per mm. scale deflection.

Two attempts were made to determine the heat capacity of the calorimeter plus reaction cell utilizing the heat solution of pottasium bromide. However due to the difficulities in designing a cell that would fit the calorimeter while permitting complete solution of the pottasium bromide, these attempts were not successful. A calculation of the heat capacity of the calorimeterplus reaction cell was made using the results from a number of determinations. These calculations led to an average value of 111 cal./°C. Of this value approximately 80 cal./°C were due to the calorimeter plus oil and 30 cal./°C to the reaction cell plus water. This division is based upon an estimate of the average weight of glass in the cell and of the water used.

Heat of Wetting

The heats of wetting of keratin, equilibrated with various amounts of water, both adsorbed and desorbed, are given in Table 1 and Figure 3. The measurements of Hedges (13) and Bright et al. (4) are included.

The water content is given in percent of the dry weight of keratin. The heats of wetting are given in calories per gram of dry keratin.

There is a rather appreciable scatter of the values of dry samples. This we feel is due to sample preparation and the difficulty of removing the last vestiges of water from the wool rather than to the calorimetry. The size of this effect could be considerable as can be seen from the steepness of the heat of wetting curve at low moisture contents.

Heat of Sorption, ΔH and $\overline{\Delta H}$

Using the method of Dole and McLaren (9), modified slightly, the net integral heats of adsorption, ΔH in calories per 100 gm. of wool keratin (above the heat of condensation of water vapour to liquid) have been calculated.

In our experiments the heat of wetting, q_o, with excess liquid water, of 100 gm. of dry wool keratin is evolved by Process 1.

(1) Protein (dry at $p = 0$) + excess liq. H_2O (at p_o) = Protein (with excess liq. H_2O at p_o) $+q_o$ cal. p_o is the vapour pressure of pure water at 24.5°C.

The heat of wetting, q, by excess liquid H_2O, of 100 gm. of keratin which has been equilibrated at pressure = p with n moles of H_2O, is given by Process 2.

(2) Protein (with n H_2O at p) + excess liq. H_2O (at p_o) = Protein

TABLE 1

Heats of Wetting of Wool Keratin by H_2O

Water Content, %		Heats of Wetting, Cal/gm of Dry Wool		
Adsorbed	Desorbed	Hedges	Bright et al	Our Values
0.00				26.67
0.00				26.32
0.00		24.1		26.42
0.39				25.03
.93				23.78
1.16				22.05
1.23				21.28
1.24				20.75
1.51				20.34
1.57				20.69
2.10				19.18
3.0		18.8	17.7	
4.21				16.58
4.91				15.53
5.0			14.6	
5.6			14.2	
6.4		13.8		10.75
8.12				
9.5		10.1		
9.6			7.6	
9.91				8.38
10.1			7.8	
10.49				7.83
11.50				6.78
12.8			5.7	
13.0			5.7	
13.1		6.3		
13.24				5.29
13.50				5.42
15.0		4.7		
15.05				4.20
17.8		3.3		
17.91				2.97
18.98				2.87
21.57				1.74
24.20				1.08
25.93				0.81
28.24				0.48
29.13				0.37
31.2				0.26
33.6				0.0
	1.08			23.01
	1.53			19.16
	1.89			20.20
	3.24			17.78
	3.44			17.55
	5.35			14.10
	5.81			13.54

TABLE 1 (continued)

Heats of Wetting of Wool Keratin by H_2O

Water Content, %		Heats of Wetting, Cal/gm of Dry Wool		
Adsorbed	Desorbed	Hedges	Bright et al	Our Values
	9.24			9.24
	9.32			10.12
	12.66			6.28
	13.04			5.44
	14.97			4.44
	17.95			2.38

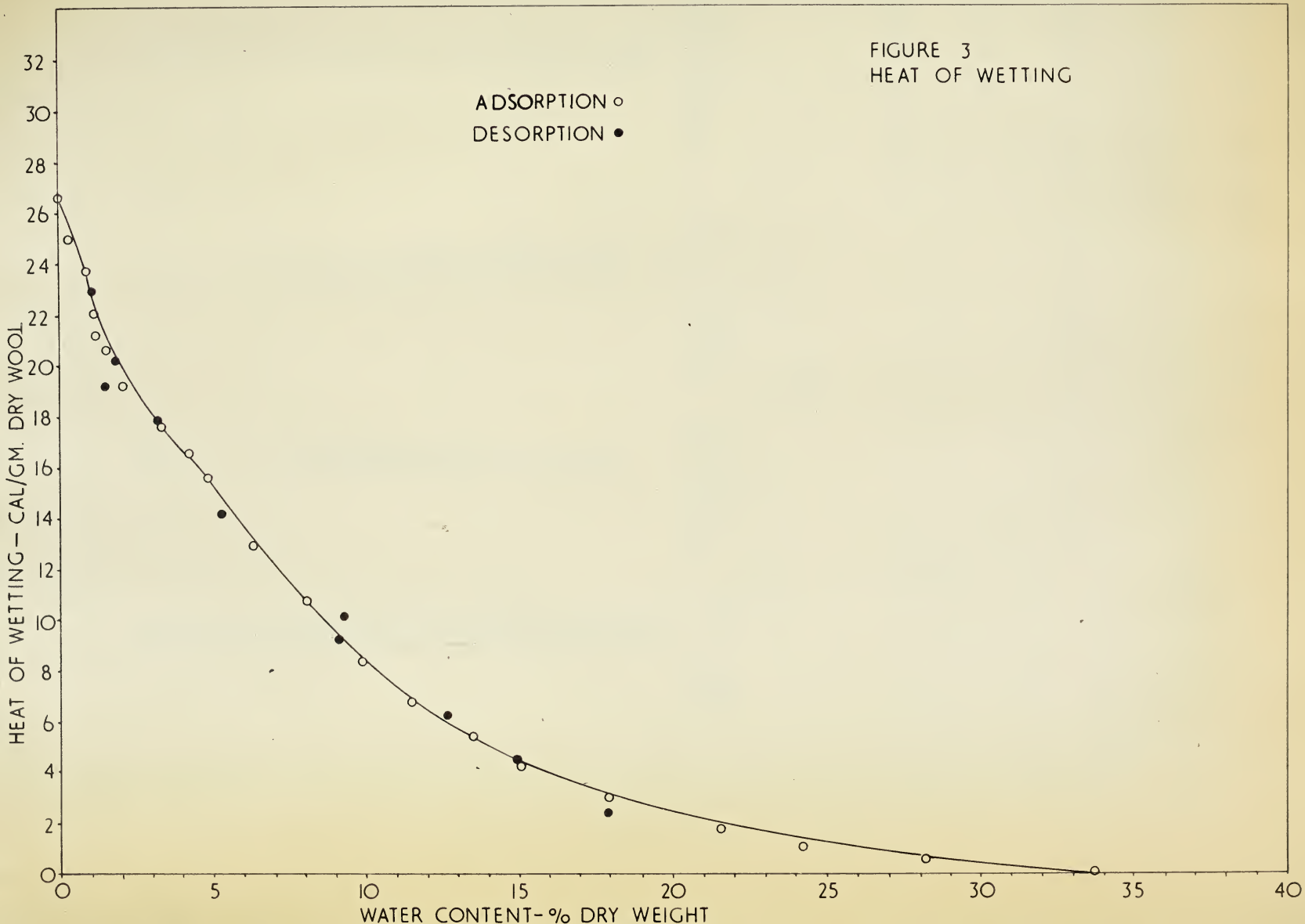


FIGURE 3
HEAT OF WETTING
ADSORPTION ○
DESORPTION ●
HEAT OF WETTING – CAL/GM. DRY WOOL
WATER CONTENT – % DRY WEIGHT
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
32
0
5
10
15
20
25
30
35
40

(with excess liq. H_2O at p_o) + q cal.

Note: The (n H_2O at p) term of the first term is now contained in the (excess liq. H_2O) of the last.

The net integral heat of adsorption, $\Delta H = q_o - q$, is obtained by subtracting Process 2 from Process 1 giving Process 3.

(3) Protein (dry at $p = 0$) + n H_2O (at p_o) = Protein (with n H_2O at p) + ΔH.

The values for ΔH, given in cal./100 gm. of dry wool are shown in Table 2 and Figure 5.

Table 2

Heat of Adsorption of Water on Wool Keratin

n, moles of H_2O/100 gm wool.	Heat of wetting cal/100 gm. wool	$-\Delta H$, cal/ 100 gm. wool	$-\overline{\Delta H}$, $= \frac{\partial(\Delta H)}{\partial n}$
0.00	2670		9720
.025			8240
.05	2270	400	6420
.075	2120	550	5350
.10	2010	660	4200
.125	1915	755	3345
.15	1835	835	2975
.20	1700	970	2520
.25	1570	1100	2515
.30	4150	1220	2545
.35	1330	1340	2400
.40	1210	1460	2400
.50	975	1695	2215
.60	770	1900	1970
.70	590	2080	1530
.80	460	2210	1045
.90	365	2305	820
1.00	295	2375	655
1.10			575
1.20	185	2485	565
1.30			440
1.40			335
1.50	65	2605	280
1.70			165
1.90	0	2670	140

The differential heats of adsorption, $\overline{\Delta H}$, ie: the partial molal heats of adsorption (Table 2 and Figure 6) were obtained by graphical and tabular differentiation of ΔH versus n. (The results of the two methods agreed very well). This corresponds to the heat of adsorption of 1 mole of water on an infinite amount of keratin, that is, an amount of keratin large enough so that the moisture content remains unchanged.

Using the data which Dr. Morrison obtained for the water vapour adsorption isotherm of wool keratin (Table 3 and Figure 4) the integral and differential free energies, ΔF and $\overline{\Delta F} = dF/dn$, for the isothermal adsorption process were calculated.

Originally Bull (5) using equation

$$\Delta F = \frac{-RT}{M} \int_0^1 a . \frac{dx}{x}$$

where M = molecular weight of water

a = grams of water adsorbed

x = partial pressure of the water, p/p_o

calculated the free energy changes for the adsorption of water vapour on a number of proteins. The integral was evaluated graphically from a plot of a/x versus x. Dole and McLaren (9) working from the general equation for dF pointed out that the integral free energy change, ΔF, for our Process 3 involves the inclusion of the term

$$nRT.\ln(x).$$

Thus

$$\Delta F = -RT \int_0^1 n.d \ln(x) + nRT \ln(x)$$

where R = the gas constant

T = the absolute temperature

and n = the number moles of water adsorbed.

The differential free energy changes were calculated for the adsorp-

tion and desorption branches of the isotherm using the formula, given by Dole and McLaren (9)

$$\overline{\Delta F} = RT \ln(x)$$

The values calculated for ΔF are given in Table 4 and Figure 5; those for $\overline{\Delta F}$ in Table 4 and Figure 6. The subscripts 1 and 2 refer respectively to the values for adsorption and desorption.

Table 3

The Adsorption and Desorption Isotherm

$x = \frac{P}{P_o}$	a_1, gm/100gm. Keratin	a_2, gm/100 gm. Keratin
0.025	1.60	2.82
.05	2.54	4.01
.075	3.30	4.85
.10	3.96	5.53
.15	5.07	6.75
.20	6.07	7.89
.25	7.05	8.97
.30	8.02	10.00
.35	8.99	10.98
.40	9.96	11.86
.45	10.94	12.77
.50	11.95	13.73
.55	12.97	14.68
.60	14.02	15.68
.65	15.11	16.73
.70	16.22	17.84
.75	17.37	19.02
.80	18.70	20.26
.85	20.21	21.68
.90	20.33	24.0
.95	26.2	27.7
.975	29.8	30.8
1.00	36.0	36.0

FIGURE 4
WATER VAPOUR SORPTION FOR
WOOL AT 24.6° C

Table 4

Integral and Differential Free Energies

n, moles of H_2O	$-\Delta F_1$, cal.	$-\Delta F_2$, cal.	$-\overline{\Delta F}_1$, cal.	$-\overline{\Delta F}_2$, cal.
0.05	160	160		
.075	225	225	2325	
.10	285	285	2175	2700
.125			1855	2400
.15	380	420	1725	2300
.20	455	530	1465	1895
.25	520	630	1235	1650
.30	580	695	1055	1400
.35	630	760	915	1200
.40	675	820	805	1050
.45	710	870	705	925
.50	745	915	615	820
.55	770	950	550	725
.60	800	985	485	630
.65	820	1010	430	555
.70	840	1035	375	485
.75	860	1060	330	430
.80	875	1080	285	370
.85	890	1100	250	325
.90	900	1115	215	280
1.00	920	1140	150	205
1.10	930	1160	105	145
1.20	940	1170	73	100
1.30	945	1175	52	70
1.40	950	1180	37	50
1.50	955	1185	25	35
1.70	960	1190	9	
1.90	960	1195	2	

Entropy Changes

The corresponding integral and differential entropy changes are given, respectively, by

$$\Delta S = \frac{\Delta H - \Delta F}{T}$$

$$\overline{\Delta S} = \frac{\overline{H} - \overline{\Delta F}}{T}$$

The values of ΔS are given in Table 5 and Figures 5; those of $\overline{\Delta S}$ in Table 5 and Figure 6. Values for the adsorption and desorption are again indicated by the subscripts 1 and 2, respectively.

Table 5

Integral and Differential Entropy Changes

n, moles of H_2O	$-\Delta S_1$, e.u.	$-\Delta S_2$, e.u.	$-\overline{\Delta S}_1$, e.u.	$-\overline{\Delta S}_2$, e.u.
.05	0.81	0.81		
.075	1.09	1.09	10.15	
.10	1.13	1.13	6.80	5.06
.125			5.00	3.18
.15	1.52	1.40	4.20	2.27
.20	1.72	1.48	3.54	2.10
.25	1.95	1.58	4.29	2.91
.30	2.15	1.76	4.60	3.44
.35	2.38	7.95	4.98	4.03
.40	2.63	2.15	5.35	4.54
.50	3.18	2.62	5.37	4.68
.60	3.70	3.07	4.98	4.52
.70	4.14	3.51	3.88	3.52
.80	4.48	3.83	2.72	2.44
.90	4.71	4.00	2.03	1.81
1.00	4.89	4.16	1.70	1.51
1.10	5.02	4.30	1.58	1.44
1.20	5.20	4.42	1.65	1.56
1.30	5.32	4.57	1.30	1.24
1.40	5.40	4.67	1.07	0.96
1.50	5.54	4.76	0.86	0.82
1.70	5.65	4.87	0.52	
1.90	5.75	4.96	0.46	

Adsorption Areas

Application of the Brunauer, Emmett and Teller equation and of the Harkins-Jura equation to the adsorption branch of the isotherm led to calculated monolayer contents, of 7.05 gm.H_2O/100 gm. wool and 7.46 gm., respectively. Dunford and Morrison have shown (11) that, though these two equations apply to opposite ends of the adsorption isotherms of water vapour on proteins as measured by Bull (5), they give about the same monolayer content. For our adsorption isotherm on wool the BET equation applies over the relative pressure range of 0.08 to 0.35 and the H-J equation over the relative pressure range $x = 0.62$ to $x = 0.90$.

Table 6 shows the monolayer contents for wool from our isotherm,

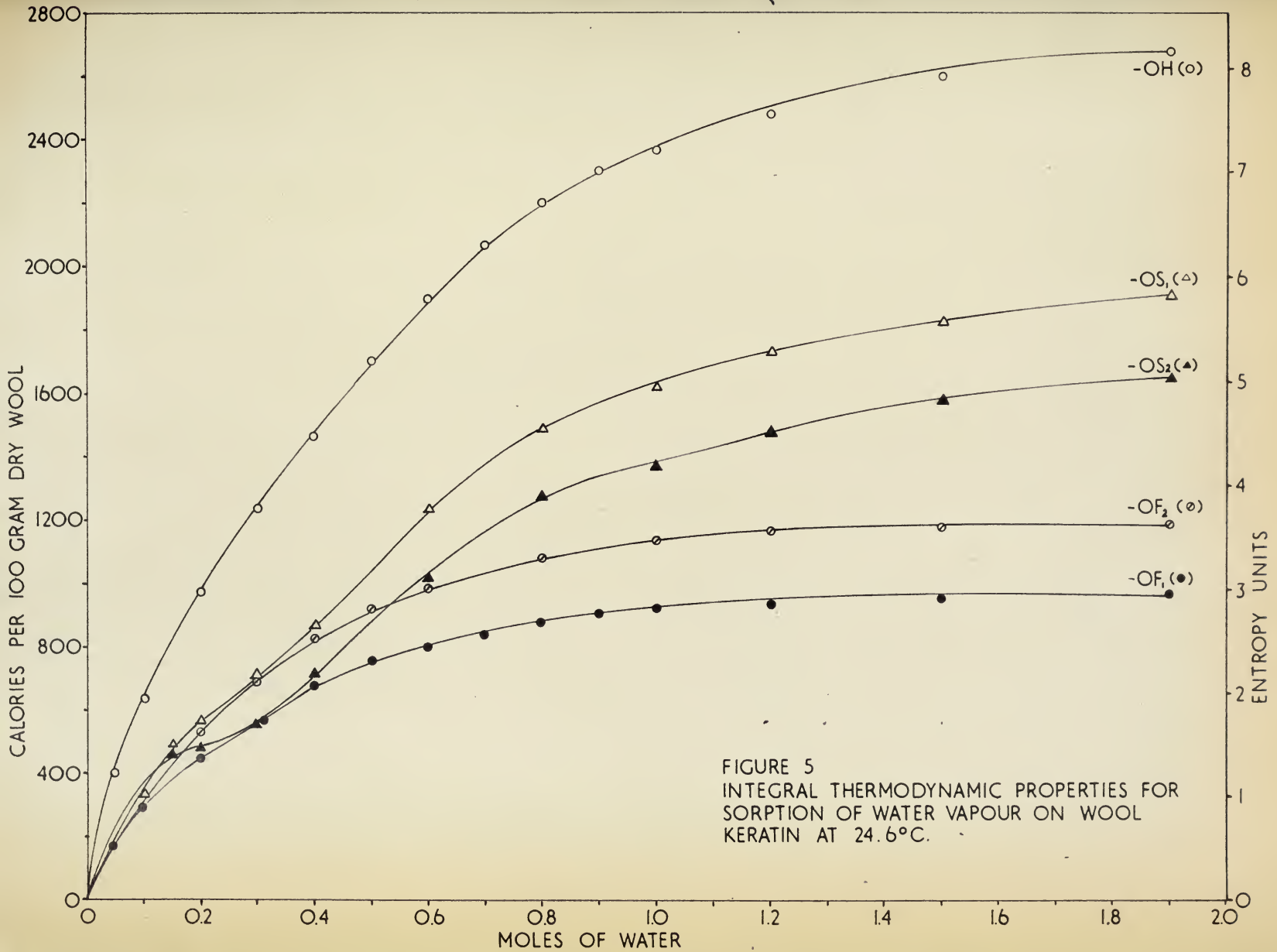
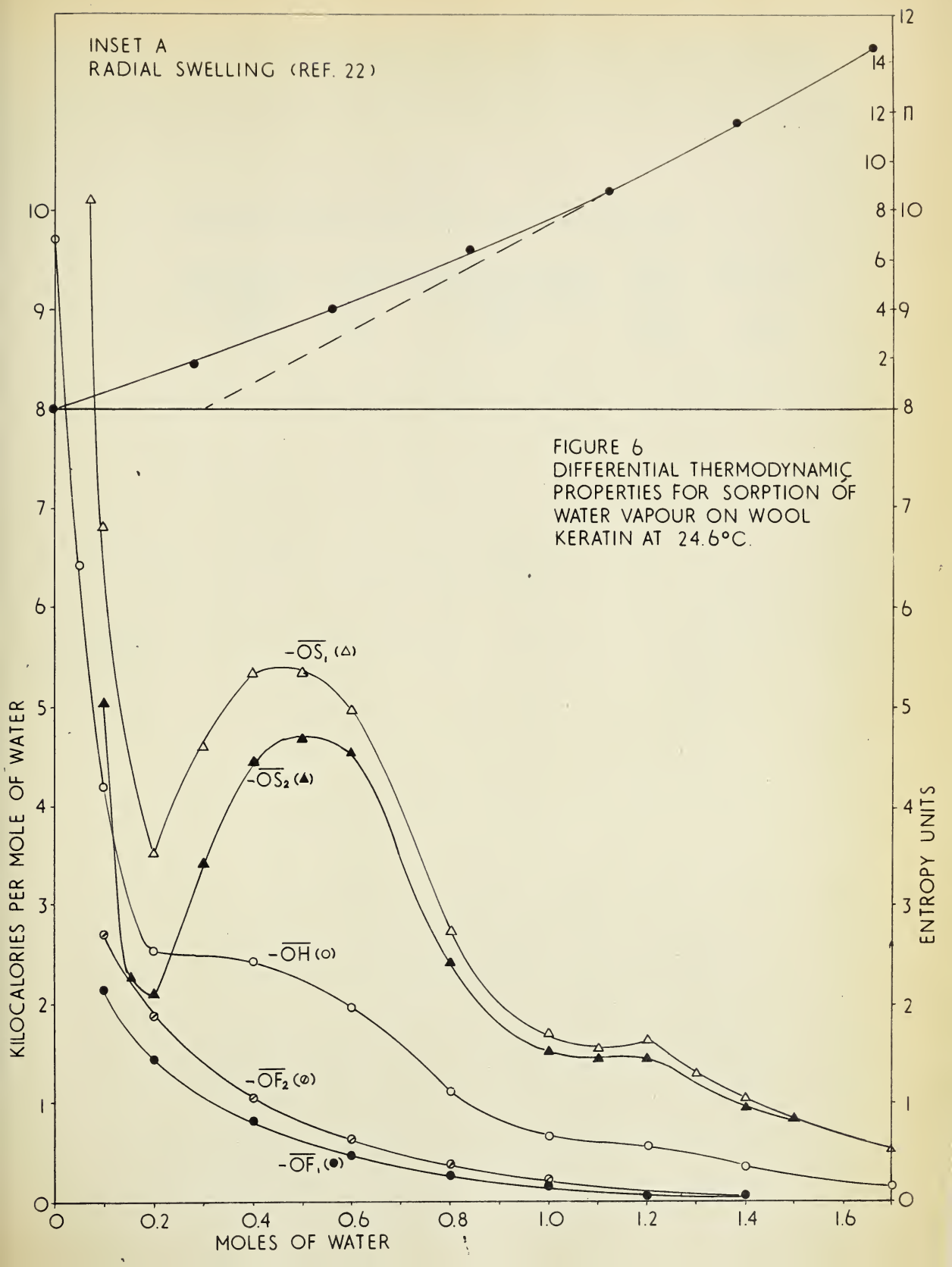


FIGURE 5
INTEGRAL THERMODYNAMIC PROPERTIES FOR SORPTION OF WATER VAPOUR ON WOOL KERATIN AT 24.6°C.


INSET A
RADIAL SWELLING (REF. 22)
FIGURE 6
DIFFERENTIAL THERMODYNAMIC PROPERTIES FOR SORPTION OF WATER VAPOUR ON WOOL KERATIN AT 24.6°C.
KILOCALORIES PER MOLE OF WATER
ENTROPY UNITS
MOLES OF WATER
$-\overline{OS}_1$ (△)
$-\overline{OS}_2$ (▲)
$-\overline{OH}$ (○)
$-\overline{OF}_2$ (⌀)
$-\overline{OF}_1$ (●)
π

that of Bull (5) and of Speakman and Cooper (17); for silk fibroin (Dunford and Morrison (11, 12) and for cellulose calculated from Wahba's data (18, 19).

Table 6

Monolayer Contents for Wool, Silk, and Cellulose

	Wool			Silk	Cellulose
	Our Values	Bull	Speakman & Cooper		
B. E. T.	7.05	6.65	6.60	4.49	3.080
H-J	7.46	7.10	7.14	4.48	3.085

DISCUSSION OF RESULTS

The heat of wetting values which we obtained (Table 1 and Figure 3) are comparable to those of Hedges (13) and Bright et al. (4). No hystersis in the heat of wetting curve for adsorbed and desorbed samples was observed.

High energy requirements to transfer the water uniformly into the body of the wool keratin might account for the scatter of values for the heat of wetting at low water contents. This scatter could probably be reduced by using smaller samples and allowing a longer time of avacuation.

On comparision with the thermodynamic functions calculated by Davis and McLaren (8) from Bull's data, our integral heats and entropies as well as the differential heats and entropies, reach higher values.

The magnitude of the differential heats of adsorption, $-\overline{\Delta H}$, are such as to suggest hydrogen bonding. Assuming an energy of 4.5 kcal. per hydrogen bond in liquid water, the bond energy for the chemisorbed water on dry wool is 9.3 kcal. per bond if each water molecule forms 2 bonds. At 2.4 kcal. the $-\overline{\Delta H}$ curve levels off following a very rapid decrease from the high values noted above. This rapid initial decrease in $-\overline{\Delta H}$ can be partly ascribed to energy required for inter-molecular swelling and to separate the subfibrils of keratin which are the smallest strutural units (1). Since this levelling coincides nearly with the end of the BET monolayer range the hydrogen bond energy is now 6.9 kcal. per bond on the assumption that molecule of water makes one bond.

From this point the $-\overline{\Delta H}$ curve continually decreases as less and less tightly bound water continues to condense on the wool, finally reaching very low values at saturation when the sucessive layers become indistinguishable from the liquid water.

The hypothesis of tightly bound chemisorbed water, resulting in a high degree of ordering of the water molecules, at low water contents is further supported by the high values of the $-\overline{\Delta S}$ curve initially. As Davis and McLaren (8) point out the entropy of freezing for water is 5.4 e.u. at 30°C. As more water is adsorbed on the wool the high $-\overline{\Delta S}$ of the ordered H_2O is decreased largely due to the disordering of the intercellular structure of the wool. This disordering will become more and more significant as reflected in the rapid decrease of the $-\overline{\Delta S}$ curve (and in $-\Delta H$). After a large part of the swelling of the fibrils has taken place the two effects, ie: ordering of the water and disordering of the gel. nearly counterbalance each other resulting in a minimum at about n = 0:2. Beyond this minimum the $-\overline{\Delta S}$ curve rised to a maximum. During the rise the monolayer is being completed resulting in an ordering effect on the water greater than the disordering of the gel of which the largest part has been accomplished. The position of the maximum nearly coincides with the water content corresponding to a completed first layer. Without this swelling phenomenon the $-\overline{\Delta S}$ should show a continued decrease from n = 0--0.6 (the beginning of the H-J region).

Beyond this to 20% water content, $-\overline{\Delta S}$ decreases as the second layer is built up since the ordering effect is diminished. Also some further swelling takes place. In this portion the water is probably condensing in the intersubfibrillar interstices.

At approximately 20% water content there is another break in the $-\overline{\Delta S}$ curve. This corresponds to a water content just after the beginning of the Harkins-Jura region, which applies to condensed films and would be considered to involve a decrease in $-\overline{\Delta S}$. However at this point the nature of the swelling curve changes. (Inset A, Figure 6)

Thus there are three ranges over which the nature of the swelling shows significant variations as reflected in the breaks in the $-\overline{\Delta S}$. From

n = 0-0.45 the first monolayer is completed. In this range the swelling is most significant in the region n = 0-0.2. The second region is that of the formation of the second layer, n = 0.45-0.90 (approximately 20% water content). From this point to saturation multilayers are being built up either as a condensed film or in clusters. This can be interpreted as indicating that up to n = 0.90 water is being compressed by adsorption within the molecular chains. Above 0.90 swelling is occurring between the fibrils and so water is not compressed.

The hysteresis in $-\overline{\Delta S}$ curve is a reflection, of course, of the hysteresis in the isotherm and consequently in ΔF. Barkas (3) in an extension of calculations presented by Porter (16), presents an explanation of the hysteresis which appears in the adsorption isotherm of isotropic gels. Although wool should be considered anisotropic due to its rather elaborate structure Mellon, Korn and Hoover (15) have shown that destruction of this structure does not affect the nature of the isotherm. According to Barkas the natural restraints imposed on a gel by swelling due to adsorption results in the introduction of changing shear stress terms due to the changing rigidity of the gel. The rather complex calculations he presents are based on the assumption that the gel is plastic and that so are some of its internal properties such as rigidty. This is supported by data presented by Alexander and Hudson (1). An equation is given by Barkas which relates the amount of adsorption with these internal factors; a shift in partial pressure for a sample which has been saturated and then desorbed will result. Since the partial pressure of water on the adsorption is higher than on the desorption branch, the affinity for water, ie: $-\Delta F$ (and $-\overline{\Delta F}$), will be lower and $-\Delta S$ (and $-\overline{\Delta S}$) will be higher than the corresponding values for desorbed samples.

The absence of hysteresis in the ΔH curve can, we believe, be explained. The larger part of the $-\Delta H$ term arises from the sorption of water

vapour on the wool with the formation of hydrogen bonds. The amount of this will be the same for adsorbed and desorbed samples. The heat of swelling term which might be expected to show hysteresis due to the above-mentioned plasticity of the swollen keratin is significant only in the range of lower water contents, since it is here that the adsorption results in the rupturing of inter-molecular hydrogen bonds. However, this is precisely the region where the swollen keratin begins to recover its structure; therefore this effect would be kept to a minimum.

As can be seen from Table 6 wool keratin has about a 10 per cent larger monolayer content by the H-J than by the B. E. T. equation whereas silk and cellulose give similar contents. This plus the fact that the H-J equation does not fit above a relative pressure of 0.90 suggests that at high water contents the water is clustering rather than forming a continuous film on the wool surface thus giving a larger apparent monolayer content. This is supported by the fact that wool is not spontaneously wetted by water whereas silk and cellulose both are.

SUMMARY

The determination of the thermodynamic properties for the adsorption of water on wool keratin provides a powerful insight into the structural changes taking place and into the energetics of the reaction. The magnitude of the energy requirements suggests, as indicated above, chemisorption.

The considerations appear general for fibrous proteins. Similar results were obtained for silk fibroin by Dunford and Morrison (10,12). Reinterpretation of those results produced conclusions similar to these for wool keratin. For both silk and wool, the swelling of the protein adsorbent has a more significant effect than previously realized.

It may be possible to estimate the actual entropy contribution of the swelling of the wool. With a knowledge of the overall $-\overline{\Delta S}$ change for the reaction and of the general nature of the $-\overline{\Delta S}$ curve for the water alone, the $-\overline{\Delta S}$ values for the wool could be obtained by difference.

It is felt that these considerations may be general for all insoluable fibrous organic materials, such as, for example, cellulose. Preliminary work on cellulose gave results fot the heat of wetting that increased as the sample weight decreased. This would indicate that the water is very tightly bound on cellulose at least at low water contents.

This appears reasonable when the large number of hydroxly groups on cellulose are taken into account. Of the 18 samples of dry cellulose whose heats of wetting were measured, it was found that heat of wetting increased from approximately 10 cal/gm. for large samples, which is comparable with the values obtained for air dryed samples (10), up to 14 and 15 cal/gm. for were dryed for periods of 2 days. In order to minimize the "zippering"

effect (2,10) these samples were vacuum dryed at room temperature.

It can be seen that the problem of obtaining uniform completely dry samples of cellulose will entail some difficulty. What the subsequent effect of this removal of the last vestiges of water from the cellulose will be, remains to be seen.

APPENDIX 1

Details of Calorimeter Construction

1. A eutectic solder consisting of 36 gm. of lead and 64 gm. of tin was used throughout the electrical portions of the apparatus.
2. The resistance wire for the heater was taken from a 100 ohm Leeds and Northrup resistor. (695 cm. of No. 32 B & S Manganinwire)
3. The lucite frame for the heater was approximately 1 inch in diameter and about one and one-quarter inches high.
4. The lucite frame for the thermel was 5.5 inches high, 3/8 of an inch wide and 3.25 inches deep, and grooved at 1/4 inch intervals to hold the thermocouple junctions.
5. The timing device was prepared by placing a single-pole double throw switch in the power lead to the Time-it (made by Precision Scientific Co.) and synchronizing this switch with the current leads to the heater.
6. A mercury commutator type switch was used in the thermocouple circuit in order to minimize contact potential. This switch also had provision for a 10,000 ohm resistor in order to protect the galvonometer when the temperature differential between the calorimeter and the bath was large.
7. In order to eliminate any e.m.f. arising from friction due to the belt driving the stirrers, a brass strip was placed so as to brush the belt; this was in turn grounded to a water pipe.
8. The table on which the Wheatstone bridge and the calorimeter assembly were mounted was covered with a grounded sheet of galvanized iron, in order to reduce any stray currents arising from thermal or contact potential sources.

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